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JOURNAL OF BACTERIOLOGY, Oct. 1981, p. 91-100
0021-9193/81/100091-10$02.00/0

Vol. 148, No. 1

Distribution of Bacteriophage φ3T Homologous Deoxyribonucleic Acid Sequences in *Bacillus subtilis* 168, Related Bacteriophages, and Other *Bacillus* Species

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Received 10 October 1980/Accepted 12 June 1981

The *Bacillus subtilis* 168 chromosome was found to share extensive homology with the genome of bacteriophage φ3T. At least three different regions of the bacterial genome hydridized to ribonucleic acid complementary to φ3T deoxyribonucleic acid (DNA). The thymidylate synthetase gene, *thyA*, of *B. subtilis* and the sequences adjacent to it were shown to be homologous to the region in the φ3T DNA containing the phage-encoded thymidylate synthetase gene, *thyP3*. SPβ, a temperate bacteriophage known to be integrated into the *B. subtilis* 168 chromosome, was demonstrated to be closely related to φ3T. Other regions of the bacterial genome were also found to hybridize to the φ3T probe. The nature and location of these sequences in the bacterial and phage chromosomes were not identified. It was shown, however, that they were not homologous to either the *thyP3* gene or the DNA surrounding the *thyP3* gene. The chromosomes of other *Bacillus* species were also screened for the presence of φ3T homologous sequences, and the *thyP3* gene was localized in the linear genomes of phages φ3T and ρ11 by heteroduplex mapping. It is suggested that the presence of sequences of phage origin in the *B. subtilis* 168 chromosome might contribute to the restructuring and evolution of the viral and bacterial DNAs.

In general, *Bacillus* species contain a substantial number of temperate phages, and many of the strains are known to be polylysogens. They also harbor defective phages and genes which are expressed when the cells are subjected to mitomycin C or UV light (1, 11, 20, 25, 33).

In most cases, the biological function of the inducible genes is not known, nor is it known why they have been retained in the bacterial chromosomes during evolution. The defective phages and cryptic genes have recently been the subject of intensive study. Some of the functions expressed in the induced cells include modification methylases and DNA repair enzymes (13, 38). In certain instances, the bacterial lysogens are known to benefit from functional complementation by phage genes. In particular, an interesting case is the host-temperate phage relationship observed between *Bacillus subtilis* and two of its temperate phages, φ3T and ρ11 (5, 35). These phages carry structural genes for thymidylate synthetases, which can complement a thymine deficiency in their host. These genes are designated *thyP3* and *thyP11*, respectively (12). The phages integrate into the *B. subtilis* chromosome at sites different from the two loci, *thyA* and *thyB*, encoding bacterial thymidylate synthetases (39). The *thyP* genes are expressed during lysogeny (35). They can also be introduced into *B. subtilis* by DNA-mediated transformation, which does not require the successful integration of the entire prophage (39). Thus, lysogenic infection and DNA-mediated transformation appear to utilize different pathways for uptake of genes into the cells. As a preliminary step in the exploration of the mechanism by which the *thyP3* gene transforms bacterial auxotrophs, the *B. subtilis* 168 chromosome was analyzed for the presence of DNA sequences homologous to the φ3T genome. The relationship between the *thyP3* gene of φ3T and the *thyP11* gene of ρ11 was also examined. To generalize my observations, the genomes of several other *Bacillus* species were screened for the presence of sequences homologous to the *thyP3* gene.

MATERIALS AND METHODS

Bacterial strains. Plasmids pFT23, -33, -401, and -502 (7) and pFT *thyP3* were propagated in *Escherichia coli* strain W5543 (*hsdR hsdM leu thi thy rpsL trp tonB2*) or W5545 (*hsdR hsdM*$^+$ *thr leu thi supE44 rpsL lac tonA pro*). *B. subtilis* strains SB168 (*trpC2*),

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SB1200 (*ilvA8 thyB1 citB1 gapA2*), and SB591 (*thyA thyB*) and *Bacillus* strains SB510, SB511, SB512, SB513, SB514, SB515, SB519, SB522, SB727, SB730, SB734, SD1096, SB1098, SB1099, SB1100, and SB1110 were from the Stanford University collection. Strain SB1207 (*leu met thr* SPβ^-) was obtained from S. A. Zahler, and strain SB1213 (*trpC2*) is a SB168 derivative which was cured spontaneously of SPβ DNA sequences but was still resistant to infection by SPβ.

Phage stocks. ϕ3T DNA was prepared after mitomycin C (Sigma Chemical Co.) induction of *B. subtilis* as outlined below. One colony of an SB168 derivative strain, cured of SPβ and lysogenized with phage ϕ3T, was inoculated into 50 ml of L broth supplemented with 10^{-5} M $MnCl_2$. The overnight-grown culture was diluted 10 times into the same medium and incubated with shaking at 37°C until early log phase (40 Klett units). At this stage, 0.5 μg of mitomycin C per ml was added, and the cells were incubated at 37°C until lysis occurred (2 to 3 h). The culture was briefly centrifuged (20 min at 8,000 rpm) to remove bacterial debris. The lysate was filtered, incubated with DNase (5 μg/ml) for 20 min at 37°C, and heated for 5 min at 60°C to inactivate the enzyme. The phage were pelleted by overnight centrifugation at 8,000 rpm in a Sorvall centrifuge. The pellet was suspended in 10 mM Tris–1 mM EDTA, pH 8.5. The phage-containing solution was adjusted to a density of 1.50 (refractive index = 1.3810) with CsCl and centrifuged for 40 h at 48,000 rpm. Further purification of the phage was obtained by centrifugation in a step CsCl gradient for 12 h at 37,000 rpm. The pooled fractions from several gradients were dialyzed against 3 liters of 10 mM Tris–1 mM EDTA, pH 8.5, DNA extracted with 1% sodium lauryl sulfate for 30 min at room temperature, and then extracted three times with phenol. The phage DNA was extensively dialyzed against 10 mM Tris–1 mM EDTA, pH 8.5, and stored in 4°C. The phage preparation was also examined by electron microscopy to verify that it did not contain phage particles with a morphology different from that of ϕ3T.

T4 phage was prepared as described by Kim and Davidson (15). Phage ρ11 DNA was a generous gift from J. C. Orrego.

Bacterial DNA and plasmid DNA preparation. Bacterial DNAs were prepared by a modification of the method of Klotz and Zimmer (16) as described by R. M. Harris-Warrick (Ph.D. thesis, Stanford University, Stanford, Calif., 1976). The pronase treatment was omitted. Closed circular plasmid DNAs were isolated from cleared lysates, prepared according to Clewell and Helinski (3). DNA was centrifuged in ethidium bromide-cesium chloride gradients (23). Ethidium bromide was removed by chromatography on Dowex A6 50W-XB. The DNA was separated from oligoribonucleotides by gel filtration on Bio-Gel A-15M. The plasmid DNA was extracted three times with phenol, ethanol precipitated, and dialyzed against 10 mM Tris–1 mM EDTA, pH 8.5.

Transformation bioassay in *B. subtilis*. *B. subtilis* cells were brought to competence as described by Harris-Warrick (Ph.D. thesis, 1976). The competent cells were concentrated 10-fold by centrifugation, suspended in 5% glycerol, and frozen in liquid nitrogen. Thawed competent cells were diluted 10-fold in minimal medium supplemented with 20 mM $MgCl_2$, and DNA was added in various final concentrations (0.1 to 1 μg/ml). The mixture was incubated for 60 min at 37°C with gentle shaking and then plated on selective media (Harris-Warrick, Ph.D. thesis, 1976).

Restriction enzyme cleavage of DNA. Restriction enzyme digestions were according to the specifications recommended by the vendors of the enzymes. *Bam*HI, *Sma*I, *Pst*I, *Bgl*II, *Sal*I, *Hpa*I, and *Hin*dIII enzymes were purchased from New England Biolabs, Inc.; *Eco*RI was purchased from Miles Laboratories, Inc. A two- to threefold enzyme excess was used routinely for most of the experiments. Reactions were terminated by heating the samples at 65°C for 7 min.

Agarose gel electrophoresis. Electrophoresis of DNA was done in 0.7% agarose horizontal slab gels (14 by 20 by 0.3 cm) at 60 V overnight or at 175 V for 3 to 4 h. Paper wicks connected the gel to tanks containing 0.089 M Tris–0.002 M EDTA–0.089 M borate, pH 8.5. The gels contained 1 μg of ethidium bromide per ml.

DNA was visualized by UV illumination of the gels, and the fluorescent bands were photographed by using a yellow filter (Kodak no. 9 Wratten filter) and Polaroid film (type 55P/N). Measurements of the molecular weights of the DNA molecules were made relative to ϕ3T DNA cleaved by *Eco*RI (7) and SPP1 DNA cleaved by *Eco*RI (10).

Nucleic acid hybridization. Hybridization experiments were carried out essentially as described by Southern (29). The hybridizations were done at 37°C in a solution containing 50% formamide, 0.75 M NaCl, and 0.075 M sodium citrate. ^{32}P-labeled complementary RNA (cRNA) was used as the probe. It was synthesized on plasmid and chromosomal DNA templates in a reaction mixture containing 50 to 100 μl of [α-^{32}P]GTP (1 mCi/ml), 0.5 mM each ATP, CTP, and UTP, 2 μg of DNA, 40 mM Tris (pH 8), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, and *E. coli* core RNA polymerase in a total volume of 100 μl. RNA polymerase was a gift from D. Brutlag. The reaction was carried out at 37°C for 3 h. DNase was then added to 20 μg/ml together with carrier RNA. The reaction was terminated by phenol extraction. [^{32}P]cRNA was separated from unincorporated nucleoside triphosphates by Sephadex G-50 chromatography. A total of 5×10^5 to 5×10^6 cpm of ^{32}P-labeled cRNA probe was used per 2 ml of hybridization solution containing a nitrocellulose filter (8 by 2 cm). After hybridization, the washed filters were blotted dry and autoradiographed at −90°C, using Du Pont Cronex 4 or Kodak XR-5 X-ray film and Du Pont Cronex Lightning Plus intensifying screens.

Electron microscopy. Preparation and spreading of heteroduplex molecules was done by the method of Davis et al. (4), except that the molecules were spread on water. No DNA extraction on ϕ3T or ρ11 phage was performed before using the phage in heteroduplex experiments. The photographs were taken at a magnification of ×4,500. The lengths of molecules were determined by measuring projections of 35-mm negatives on the surface of a Hewlett-Packard 9864 A digitizer interfaced with a Hewlett-Packard 9810A cal-

...ator. The projections were measured at a total magnification of ×135,000. All measurements were made relative to a standard DNA molecule, pSC101, mounted on the same grid. The molecular weight of the standard pSC101 DNA used in all the calculations was 5.8 megadaltons (Mdal) (28).

Cloning of *Eco*RI* fragments. A 3.5-μg amount of pFT401 was incubated with 2.5 mM $MgCl_2$, 10 mM Tris (pH 8.5), and 10 U of *Eco*RI enzyme (Miles Laboratories) at 37°C in a total volume of 50 μl. Samples of 10 μl each were removed after 1, 3, 7.5, and 25 h, and the reactions were terminated by heating the samples to 65°C for 7 min. A 0.5-μg amount of *Eco*RI*-cleaved pFT401 from each sample was mixed with 0.2 μg of *Eco*RI-cleaved pMB9 in a total volume of 70 μl. Ligation was carried out at 10 μg of DNA per ml for 1 h at 20°C, using T4 ligase and the buffer described by Sgaramella et al. (26). *E. coli* W5443 competent cells were prepared as described elsewhere (27).

The transformants (Thy^+ Tc^r) were selected on aa plates (Spizizen minimal medium [30] supplemented with 25 μg each of the common amino acids per ml, glucose, 20 μg of tetracycline per ml, and agar).

RESULTS

Construction of chimeric plasmids containing φ3T DNA sequences. Most of the recombinant plasmids (pFT23, pFT24, pFT33, pFT401, and pFT502) used in this work were constructed previously in our laboratory. Figure 1a summarizes their molecular structures. The chimeras contain different but overlapping φ3T DNA sequences joined to *E. coli* plasmid pSC101 or pMB9. A DNA segment of φ3T origin is common to all of the recombinant plasmids and complements the *thyA* thymine deficiency mutation in *E. coli*. It it also able to transform Thy^- auxotrophs of *B. subtilis* to prototrophy. Therefore, this segment of φ3T DNA is assumed to contain the *thyP3* gene. To achieve higher purification of this specific gene, it was necessary to eliminate nonessential DNA sequences flanking the *thyP3* gene. This was accomplished by subcloning of *Eco*R1* fragments (22) of pFT401 in the *Eco*RI site of plasmid pBR322. Figure 1b shows the structure of the resulting chimeric plasmid, pFT *thyP3*. The molecular size of the *Eco*RI DNA insert carried by pFT *thyP3* is 0.57 Mdal.

Sequences homologous to φ3T DNA in the *B. subtilis* chromosome. Structural analyses of DNA sequence relationships were performed by using the technique of cRNA-DNA hybridization coupled with restriction enzyme analysis (29). At least three different regions of the *B. subtilis* chromosome capable of hybridizing φ3T cRNA were identified.

DNA homology of the bacterial *thyA* and phage *thyP3* genes was demonstrated by hybridization of cRNA prepared on pFT *thyP3* to the

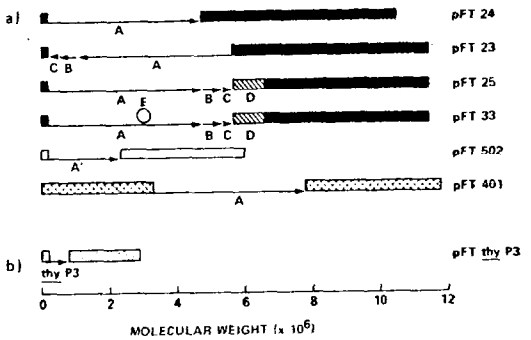

FIG. 1. *Molecular structure of chimeric plasmids. All DNAs are aligned from the BamHI site located in the vectors. The vector components of the hybrids are represented by heavy lines: ■, pSC101; ▩, RSF2122; ▨, pBR322. The inserts are shown as thin lines. There is, in all cases, an EcoRI site at the boundary between the vectors and inserts. Additional EcoRI sites are denoted as arrows. The direction of the arrows indicates the orientation of the inserts. (a) Diagrams of plasmids described earlier (7) but used in this work. The sizes (in megadaltons) of the segments are (A) 4.5, (B) 0.67, (C) 0.24, (D) 0.9, (E) 0.9, (A') 2.1. A' is homologous to left part of segment A. (b) Diagram of the chimeric plasmid constructed by cloning the EcoRI* fragment of pFT401 in the pBR322 vector. The size of pFT thyP3 insert is 0.57 Mdal.*

*Eco*RI-cleaved fragments of *B. subtilis* DNA which are associated with *thyA* biological activity. The data showing partial purification of *B. subtilis* segments containing *thyA* and *thyB* genes are presented in the accompanying paper, where it is shown that wild-type *B. subtilis* contains two *Eco*RI fragments associated with Thy^+ activity (32). The $thyA^+$ gene resides on an *Eco*RI fragment larger than 10 Mdal, whereas the $thyB^+$ gene is carried on a 4.2-Mdal *Eco*RI fragment. RNA complementary to pFT *thyP3* hybridized to only one band of the *B. subtilis* *Eco*RI restriction digest (Fig. 2). The molecular weight of the DNA in this band corresponded to the segment containing *thyA* biological activity. The size of the DNA insert in pFT *thyP3* was only 0.57 Mdal (~950 base pairs), and therefore it contained very little, if any, of the DNA sequences other than the *thyP3* gene. Thus, it is assumed that the observed hybridization occurs between the phage and bacterial *thy* genes. Control experiments involving similar hybridizations between cRNA transcribed from the plasmid vector pBR322 gave negative results.

To construct a physical map of the *thyA* region, *B. subtilis* DNA was cleaved with different restriction enzymes and then hybridized to P^{32}-labeled cRNA prepared from several of the recombinant plasmids. Only the *Hin*dIII enzyme

cleaved this region internally (Fig. 3). The difference in the hybridization patterns observed in Fig. 3a and 3b is due to the different lengths of the probes used in these two experiments. It follows that the *B. subtilis* chromosome contains one or more other genes which have homology to $\phi 3T$ sequences. This gene (or genes) was linked to *thyP3* in the phage and to *thyA* in *B. subtilis*, as evidenced by the unsuccessful attempts to separate them by digestion with the following enzymes: *Eco*RI, *Bam*HI, *Sma*I, *Pst*I, *Bgl*II, *Sal*I, and *Hpa*I (data not shown). The size of the restriction fragment that contained the *thyA* region was 7.8 Mdal for *Pst*I, 9.4 Mdal for *Bgl*II, 6.9 Mdal for *Hpa*I, and more than 10 Mdal for *Eco*RI, *Bam*HI, *Sal*I, and *Sma*I. Double digestion of *B. subtilis* 168 DNA with *Eco*RI and *Hind*III enzymes, followed by hybridization with cRNA pFT502, was compatible with the physical map of the *thyP3* homologous region shown in Fig. 4.

In addition to the segment associated with the

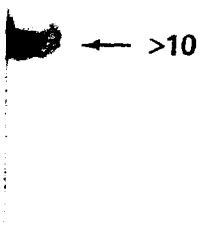

FIG. 2. *Hybridization of cRNA pFT thyP3 to EcoRI-digested B. subtilis 168 DNA. Two micrograms of SB168 DNA was digested with EcoRI enzyme, electrophoresed through a 0.7% agarose gel, transferred to a nitrocellulose filter (29), and hybridized to ^{32}P-labeled RNA complementary to plasmid pFT thyP3. The conditions of hybridization are described in Materials and Methods. Sizes of the stained DNA bands are expressed in megadaltons.*

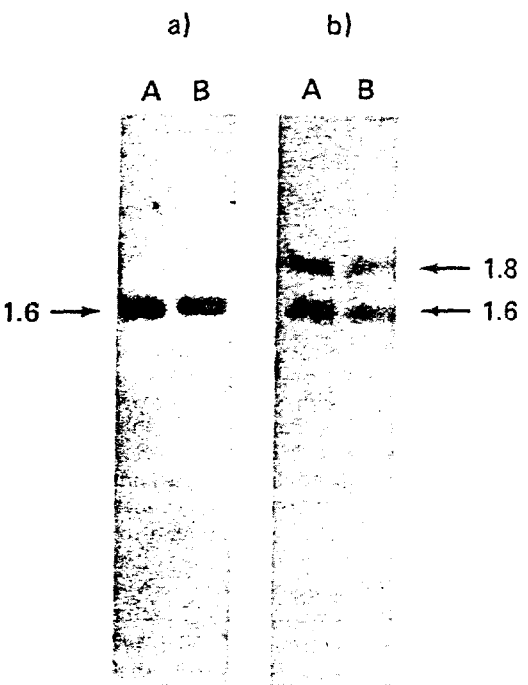

FIG. 3. *Hybridization of (a) cRNA pFT thyP3 and (b) cRNA pFT502 to HindIII-cleaved DNAs: SB168 (channel A) and SB1207 (channel B). The sizes of the stained bands are expressed in megadaltons.*

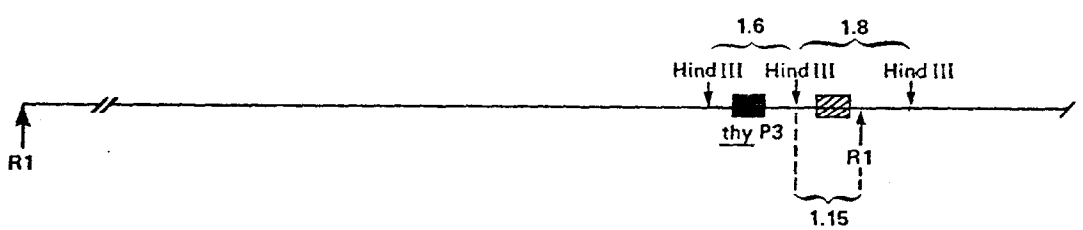

FIG. 4. *Proposed map of HindIII and EcoRI restriction sites in the thyA region of the B. subtilis chromosome (SB1200). The heavy bar represents sequences that hybridize to thyP3 DNA (0.57 Mdal). The hatched bar represents a gene or genes homologous to sequences of pFT502 other than thyP3 or pMB9 vector. Sizes of segments are indicated in megadaltons.*

thyA biological activity, several other *Eco*RI fragments of chromosomes from laboratory strains derived from *B. subtilis* 168 (30) (except SB1207 and SB1213; see below) hybridized with the following probes: cRNA pFT23, cRNA pFT24, cRNA pFT25, and cRNA pFT502. These *Eco*RI fragments were identified as cleavage products of a cryptic prophage, SPβ, which is known to be integrated into the *B. subtilis* chromosome between *ilvA* and *kauA* (40). Figure 5 presents an example of the experiment supporting this conclusion. The *Eco*RI band of 1.25 Mdal, which hybridized to cRNA pFT502 in SB168 (Fig. 5A), was missing in the *Eco*RI digest of strain SB1207 cured of SPβ (Fig. 5B) and reappeared after this strain was lysogenized with SPβ (Fig. 5C).

Approximately 20 bands appeared in the autoradiogram shown in Fig. 6 when the RNA complementary to the whole φ3T genome was used as a probe in hybridization with the restriction digest of the SPβ lysogen. The bands were stained unequally, indicating either their uneven degree of homology with the probe or their presence in more than equimolar amounts. The sum of their sizes was 52 Mdal (or 70.4 Mdal if strongly hybridizing bands are counted as doublets; see Table 1). This estimate corresponds to

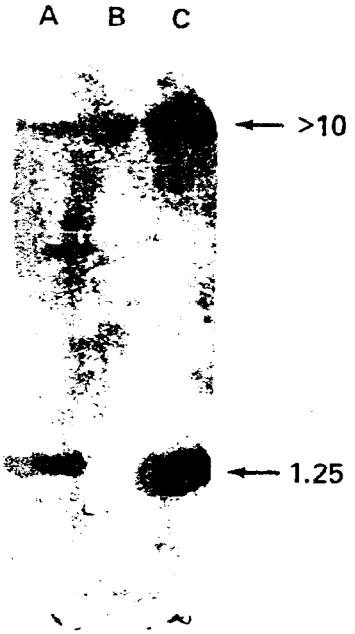

FIG. 5. *Hybridization of cRNA pFT502 to EcoRI-cleaved B. subtilis DNAs: (A) SB168; (B) SB1207; and (C) SB1207 lysogenized with SPβ. Sizes of stained bands are expressed in megadaltons.*

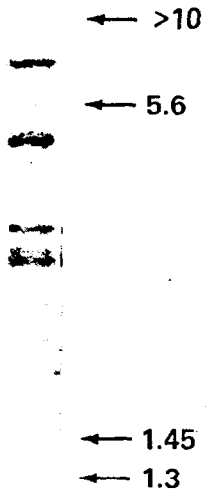

FIG. 6. *Hybridization of cRNA φ3T to EcoRI-cleaved SB168 DNA. The arrows indicate the positions of bands that appear on the autoradiogram upon longer exposure. These bands can also be detected in SPβ⁻ strains SB1207 and SB1213 (see Fig. 7).*

the size of the phage genome which has a complexity similar to the φ3T genome. SPβ contained sequences complementary to pFT502, but it did not carry the *thyP3* gene. This is revealed by the lack of its hybridization with cRNA pFT *thyP3*.

Finally, it was shown that the *B. subtilis* chromosome contained yet another region or regions homologous to the φ3T genome. The *Eco*RI fragments of the DNA from two strains independently cured of SPβ prophage (SB1207 and SB1213) hybridized to cRNA transcribed from the φ3T DNA (Fig. 7 and Table 1). The hybridization pattern was limited to four *Eco*RI bands instead of the large number of bands observed in the SPβ lysogens. The largest of these bands

TABLE 1. *Sequences homologous to φ3T DNA in the B. subtilis chromosome*

*Eco*RI fragments of *B. subtilis* 168 hybridizing φ3T cRNA[a]		*Eco*RI fragments of *B. subtilis* SB1207 and SB1213 (SPβ⁻) hybridizing φ3T cRNA[b]	
Size (Mdal)	Hybridization	Size (Mdal)	Hybridization
7.9	+++	>10	++
5.6	+	5.6	++
4.7	+++		
4.1	+		
4.	+		
3.6	+		
3.1	+++		
2.8	+		
2.6	+++		
2.3	+		
2.05	+		
1.85	+		
1.7	+		
		1.45	+
1.3	+	1.3	++
1.25	+		
1.15	+		
0.9	+		
0.63	+		
0.53	+		
52.06			

[a] If strong (+++) bands are counted as doublets, the total size of the hybridizing fragments is 70.4 Mdal. In addition to the bands listed above, *Eco*RI fragments of 1.45 and >10 Mdal appeared on the autoradiogram upon longer exposure. +, weak.

[b] The total size of the bands other than the SPβ-specific or *thyA* region DNA is 8.35 Mdal. ++, Strong; +, weak.

(>10 Mdal) corresponded to the *thyA* region. The nature and location of the other three segments of 5.6, 1.45, and 1.3 Mdal were not identified.

When the P^{32}-labeled cRNA was transcribed from a *B. subtilis* chromosome containing SPβ prophage (SB581) and hybridized to an *Eco*RI digest of φ3T DNA, not all of the bands were labeled (Table 2). The pattern described in Table 2 indicates the portions of the φ3T genome that were detected by this technique. They corresponded to the portions of the φ3T genome present in the *B. subtilis* chromosome.

Sequences homologous to φ3T in other *Bacillus* species. Table 3 summarizes the results of screening the chromosomes of several *Bacillus* species for the presence of *thyP3* homologous sequences. In most cases, the presence of the *thyP3* homologous gene was accompanied by the presence of the linked φ3T homologous sequence (*B. subtilis* Marburg, *B. globigii*, *B. subtilis niger*, and *B. coagulans*). *B. mycoides* and *B. pumilus* DNAs did not hybridize cRNA pFT *thyP3* but showed hybridization with cRNA pFT502, indicating the presence of the gene or genes linked to *thyP3*. Only *B. subtilis* Marburg and *B. coagulans* were found to contain a SPβ-specific *Eco*RI band which hybridized to cRNA pFT502 (Fig. 5). Thus, these two species are assumed to be SPβ lysogens.

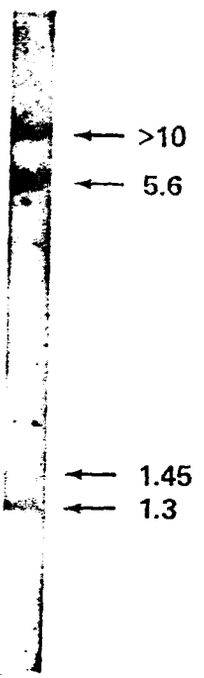

FIG. 7. *Hybridization of cRNA φ3T to EcoRI-cleaved SB1207 (SPβ⁻). An identical hybridization pattern was observed by using strain SB1213 DNA. Sizes of stained bands are expressed in megadaltons.*

TABLE 2. *Hybridization of RNA complementary to SB591 (SPβ⁺) to EcoRI-cleaved φ3T DNA*[a]

Band no.	Hybridization
1	−
2	−
3	−
4	+
5ab	+
6	+
7	+
8	+
9	−
10	+

[a] *Eco*RI bands of φ3T were numbered according to Ehrlich et al. (7). Detection of hybridization to bands smaller than band 10 was below the resolution of the technique used. +, Hybridization present; −, no hybridization.

TABLE 3. *Screening of bacterial chromosomes for the presence of the thyP3 gene and linked φ3T homologous sequence*

Strain	Hybridization of cRNA pFT *thyP3* to *Eco*RI band,[a] >10 Mdal	Hybridization of cRNA pFT502 to *Eco*RI band[a]		Transformation efficiency (no. of transformants/no. of viable cells per μg of DNA)[b]		
		>10 Mdal	1.2 Mdal	Thy	His	Phe
SB1213 (SPβ⁻ *trp-2*)	+	+	−	6×10^{-4}	3×10^{-4}	3×10^{-4}
SB522 (*B. subtilis* Marburg)	+	+	+	5×10^{-4}	3×10^{-4}	3×10^{-4}
SB519 (*B. megatherium*)	−	−	−	3×10^{-7}		5×10^{-7}
SB515 (*B. polymyxa*)	−	−	−			
SB514 (*B. subtilis* subsp. *terminalis*)	−	−	−	1.5×10^{-7}		1.5×10^{-7}
SB513 (*B. cereus*)	−	−	−			
SB512 (*B. globigii*)	+	+	−			
SB511 (*B. mycoides*)	−	+	−			
SB510 (*B. subtilis* subsp. *niger*)	+	+	−	10^{-7}		
SB1110 (*B. amyloliquefaciens*)	−	−	−			
SB1100 (*B. subtilis* subsp. *natto*)	−	+	−	1.5×10^{-4}	3×10^{-4}	3×10^{-4}
SB1099 (*B. coagulans*)	+	+	+	8×10^{-4}	6×10^{-4}	6×10^{-4}
SB1098 (*B. brevis*)	−	−	−			
SB1096 (*B. pumilus*)	−	+	−	1×10^{-3}	5×10^{-4}	5×10^{-4}
SB734 (*B. subtilis* N)	−	−	−	10^{-7}		
SB734 (*B. subtilis* H)	−	−	−			
SB727 (*B. subtilis* K)	−	−	−			
W5445 (*E. coli* K-12)	−	−	−		NT	NT
T4 phage	−	−	−		NT	NT

[a] An *Eco*RI band 2.5 Mdal from SB727 (*B. subtilis* K) hybridized both cRNA pFT *thyP3* and cRNA pFT502 probes. +, Hybridization present; −, no hybridization.

[b] The recipient strain in the transformation assays was SB748 (*thyA thyB his-2 aro-2*). Transformation efficiency is listed only when the number of transformants in the assays was significantly higher than the number of revertants for the marker tested. The numbers shown are averages of six sets of experiments. NT, Not tested.

The DNA extracted from the *Bacillus* species studied was also tested for its ability to transform the *B. subtilis* thymine auxotroph SB591 to prototrophy. In many cases, the high transformation efficiency to the Thy⁺ phenotype was correlated with the presence of the *thyP3* homologous sequences in the donor species (*B. subtilis niger, B. coagulans, B. subtilis* Marburg, and *SB1213*). In the case of DNA from *B. globigii*, which was found to hybridize the *thyP3* probe, the transformation efficiency was below the reversion frequency of the recipient strain. This indicates that the DNA sequences surrounding the *thy* genes in the donor and recipient strains are nonhomologous. An alternative explanation which proposes the existence of a *B. subtilis* restriction system that degrades *B. globigii* DNA was ruled out by the results of Harris-Warrick (Ph.D. thesis, 1976). A few strains (*B. megatherium, B. subtilis* subsp. *terminalis, B. subtilis* subsp. *natto,* and *B. pumilus*) did not hybridize to the *thyP3* probe, but their DNA still transformed the *B. subtilis* 168 thymine auxotroph to prototrophy. This might have been due to transformation with the *thyB* locus which was contained in the donor strains. Another possibility is that the *thyA* genes in the donor strains diverged considerably from the *B. subtilis thyA* sequence and therefore were not detected under the hybridization conditions used.

Neither *E. coli* nor T4 phage DNA was able to transform *B. subtilis* strain SB591 to Thy⁺ or hybridize to *thyP3* sequences. These results demonstrate specificity of the Southern hybridization technique and transformation assay used in these studies.

Mapping of the *thyP11* gene in ρ11. The *thyP3* gene has been mapped in the φ3T genome by heteroduplex analysis of hybrids formed between the chimeric plasmid pFT25 and phage DNA (7). In view of the known similarities between φ3T and ρ11 (5), I decided to use the same technique to localize *thyP11* in ρ11. The pFT24 plasmid containing *thyP3* of the φ3T phage was

linearized with the *Bam*HI restriction enzyme. After denaturation, this plasmid DNA was annealed to the complementary single strands of the ρ11 DNA (Fig. 8). A double-stranded region of homology with a size of 3.0 ± 0.2 Mdal was found at a distance of 50 to 54% of the total length from one of the termini of the ρ11 DNA molecule. The standard error of this measurement was 3% of the fractional ρ11 length. The double-stranded region found in the heteroduplex of ρ11 with segment A of plasmid pFT24 was not perfectly homologous. Two small loops could be detected. The right part of segment A (1.5 Mdal) did not hybridize to the ρ11 DNA. This indicates that the DNA sequences surrounding the *thyP* gene in the ϕ3T and ρ11 phages are different. Figure 8 shows also the localization of the *thyP3* gene in the ϕ3T genome. The *thyP3* gene was situated at a distance 44 to 48% from one of the ϕ3T ends. Statistical error of this measurement was 2% of the fractional ϕ3T length.

DISCUSSION

The results described here provide evidence for the existence of extensive DNA homologies between the chromosomes of *B. subtilis* 168 and its temperate bacteriophage ϕ3T. Three distinct regions of the bacterial chromosome were identified that were capable of hybridizing RNA complementary to the ϕ3T genome.

One of these regions contains the structural gene for thymidylate synthetase A and was shown to be homologous to the phage-encoded *thyP3* gene. In addition, another homologous DNA sequence (equal to or less than 2.15 Mdal) was located next to *thyA* in *B. subtilis* and next to *thyP3* in ϕ3T. The nature of this other gene

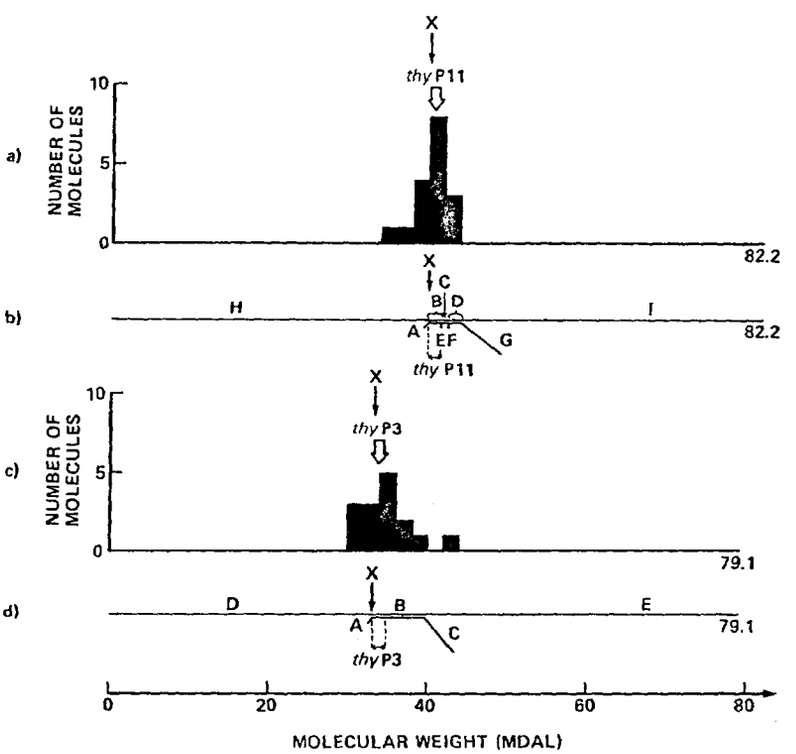

FIG. 8. *Localization of the thyP gene in ρ11 and ϕ3T. (a) Histogram of the position of point X (see below) is shown in relation to the ends of ρ11 DNA. X corresponds to the far left end of the ϕ3T insert in pFT24 and pFT25. (b) Diagram of ρ11 DNA heteroduplexed with BamHI-linearized pFT24 DNA. Segments B, C, and D represent the regions of homology between the two DNA strands. Loops E and F correspond to regions of nonhomology. The sizes of heteroduplex regions are (in megadaltons): (A) 0.2; (B) 1.73; (C) 0.11; (D) 1.1; (E) 0.13; (F) 0.26; (G) 6.39; (H) 41.1; (I) 38.8. Seventeen molecules with the structure represented by this diagram were analyzed. (c) Histogram of the position of point X is shown in relation to the ends of ϕ3T DNA. (d) Diagram of ϕ3T DNA heteroduplexed with BamHI-linearized pFT25 DNA. Segment B represents the region of homology between ϕ3T and pFT25. The sizes of heteroduplex regions are (in megadaltons): (A) 0.2; (B) 6.3; (C) 4.7; (D) 34.8; (E) 41.1. Fifteen molecules with the structure represented by this diagram were measured.*

(or genes) was not identified. No markers closely linked to *thyA* or *thyP3* have been reported in *B. subtilis* of φ3T. In other systems, such as T4 phage, DNA sequences close to the structural gene for thymidylate synthetase code for proteins involved in DNA metabolism (37). In contrast, the *E. coli* or *Salmonella typhimurium thyA* locus maps between the *lys* and *arg* loci, away from other genes involved in DNA synthesis (7).

The second region of extensive homology between the bacterial genome and the φ3T genome was identified as an SPβ prophage. SPβ is a temperate, cryptic bacteriophage present in *B. subtilis* strains derived from Spizizen's transformable strain 168 (31). The discovery by Warner et al. (36) of a cured strain has permitted the characterization of SPβ. It is a fairly large phage of complex structure similar morphologically to φ3T and ρ11 (36) but not to PBSX, a defective bacteriophage also known to lysogenize all *B. subtilis* 168 strains (25). The prophage attachment site for SPβ lies between *ilvA* and *kauA* (40). SPβ does not convert *B. subtilis* Thy⁻ auxotrophs to prototrophy upon lysogenization. It can, however, carry out a specialized transduction of the *citK* and *kauA* genes (40). Its mechanism of specialized transduction was proposed by Zahler et al. (40) to resemble closely the *E. coli* phage λ *dgal* system (2). The results described in this communication demonstrate that the phages SBβ and φ3T are closely related. When RNA complementary to the φ3T genome was hybridized to the *Eco*RI restriction digest of a SPβ lysogen, more than 20 bands (total of 50 to 80 Mdal) were homologous to the radioactive RNA probe. The restriction fragments which did not cross-hybridize between the two genomes presumably code for the traits that are different in the two phages, such as immunity. In addition, it was shown that SPβ lacks the *thyP3* sequence (0.57 Mdal), although the DNA sequence located next to this gene in φ3T is still present in the SPβ genome.

Finally, it was shown that the *B. subtilis* chromosome contains yet another region homologous to φ3T which is different from *ThyA* or SPβ DNA. Two strains independently cured of SPβ still hybridized φ3T probe. Three *Eco*RI fragments of *B. subtilis* 168 DNA (molecular sizes of 5.6, 1.45, and 1.3 Mdal) showed homology to sequences other than the *thyP3* region of φ3T. The nature and chromosomal locations of these sequences were not identified.

The presence of φ3T homologous sequences scattered at different locations in the *B. subtilis* chromosome might promote site-specific recombination and, in consequence, restructuring and evolution of the bacterial and phage chromosomes. The ability of SPβ to recombine with φ3T (described in the accompanying paper [32]) supports this hypothesis and suggests that *thy*-transducing phages such as φ3T and ρ11 could have been created during recombination events between SPβ phage and the *thyA* region of *B. subtilis*.

B. subtilis is not the only organism known to carry multiple sequences of viral origin in its genome. This has been shown to be a common property of many eucaryotic systems. Simian virus 40 (14, 17) and adenovirus (6, 9, 24) sequences are present in many copies in the genomes of their transformed mammalian hosts. The thymidine kinase gene coded by herpes simplex virus can integrate stably into mouse DNA at many different sites (21). Numerous species of vertebrates also contain endogenous latent RNA tumor viruses present in the cells as proviral DNA copies (8, 18, 23). The distribution and functions of these proviruses in the host genomes are not known at present, nor is it known whether they can promote site-specific recombination of the eucaryotic chromosomes. In view of the complexity and inherent difficulties in studying the structure of the mammalian genome, the arrangement and role of cryptic genes and phages in *B. subtilis* might be of general interest as a model system for understanding the virus-host relationship.

ACKNOWLEDGMENTS

I am grateful to J. Lederberg, in whose laboratory this work was done, for his continuous support and many helpful suggestions. I also thank him, M. Winkler, and A. T. Ganesan for critical reading of this manuscript. I am indebted to H. Bursztyn-Pettegrew for sharing her unpublished data with me and to P. Evans for excellent technical assistance.

This work was supported by Public Health Service predoctoral fellowship GM 00295 and grant CA 16896 from the National Institutes of Health.

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